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Assessment of tubule occlusion properties of an experimental stannous fluoride toothpaste: A randomised clinical *in situ* study

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ABSTRACT

Objectives: To evaluate the ability of a modified *in situ* model to differentiate dentinal tubule occlusion properties of toothpaste formulations over 10 days of treatment.

Methods: This was a single-centre, three-treatment period, crossover, randomised, single-blind study with healthy participants wearing two lower oral appliances, each retaining four dentine samples, for 10 treatment days during each period of the study. Samples were power-brushed *ex vivo* twice on each treatment day with a Test toothpaste containing 0.454% stannous fluoride, a Control fluoride toothpaste containing 0.76% sodium monofluorophosphate, or mineral water. Dentine samples were subjected to *in situ* acid challenge (orange juice) on Days 9 and 10. Scanning electron microscopy images obtained at baseline and after 1, 4, 8 and 10 days of treatment were graded for degree of surface coverage by four calibrated examiners; the primary study endpoint was Day 8.

Results: After 4, but not 8, days' treatment, the degree of tubule occlusion increased in the dentine samples treated with the Test or Control toothpastes compared with the water-treated samples ($p < 0.01$ and $p < 0.05$, respectively). Following the acid challenge (Day 10), there was a statistically significantly greater degree of occlusion in the Test toothpaste-treated dentine samples compared with those treated with water ($p < 0.01$). No other comparisons were statistically significant. All study treatments were generally well-tolerated.

Conclusions: This modified *in situ* model was unable to demonstrate statistically significant between-treatment differences in dentinal tubule occlusion after 8 days. Conversely, there are recognised developments that could be made to better identify product differences. Clinicaltrials.gov: NCT02768194.

Clinical significance: Dentine hypersensitivity can be managed through brushing with stannous fluoride toothpastes, which occlude patent dentine tubules. Clinical studies measure pain but *in situ* models are needed to demonstrate occlusion intra-orally. However, this study did not demonstrate superior occlusion with stannous toothpaste; further methodological development is required to investigate its mode of action.

1. Introduction

Dentine hypersensitivity (DH) results primarily from exposure of patent dentinal tubules due to gingival recession and/or enamel loss (*e.g.*, through erosion or abrasion) [1–3]. The characteristic short, sharp pain of DH is thought to result from an external stimulus, such as a temperature or osmotic differential, causing fluid movement within exposed dentinal tubules, which in turn stimulates nerve processes in the pulpal area of the dentine [4,5].

Based on its presumed aetiology, management of DH with home-use oral-care products may be approached either by depolarisation of the afferent nerve membrane to block the pain response or by physically occluding the dentinal tubules, thus reducing dentinal fluid movement and pulpal nerve irritation [6,7]. Tubule-occluding agents, such as strontium and stannous salts, bioglasses, silicas or oxalates [8–13], seal or block the dentinal tubules by precipitating insoluble materials onto the dentine surface and/or within the dentinal tubules, thereby reducing the effect of external stimuli on dentinal fluid movement [14]. Some evidence suggests that occlusion-based agents may provide faster relief of DH than those based on nerve depolarisation [15].

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Stannous fluoride (SnF_2) has been incorporated into oral hygiene products since the 1960s [16] and there is substantial published clinical evidence that SnF_2-containing toothpastes can reduce DH [8,11,17]. SnF_2 is believed to provide relief from DH by occluding dentinal tubules through chemical precipitation of tin oxides and hydroxides onto the dentine surface, with other salivary constituents and particulates from the formulation [18,19]. This precipitate has been shown *in vitro* to be relatively resistant to solubilisation by dietary acids [20]. Stannous ions in neutral aqueous solution tend to oxidise to the stannic state and form hydroxide precipitates. This can be avoided by formulating the ion in non-aqueous, glycerin-based toothpaste formulations that have similar viscosity/flow characteristics to conventional aqueous products. Such products release the stannous ion on dilution in saliva during use [11]. Based on *in vitro* data, a formulation of a marketed anhydrous SnF_2 toothpaste with a modified polymer system based on polyacrylate (carbomer) in a glycerin-polyethylene glycol non-aqueous liquid phase has recently been developed; this allows more rapid and complete deposition of occluding precipitates [21].

The ability of this new formulation to relieve DH is being assessed in a comprehensive programme of clinical trials [22–24]. An *in situ* model has been developed for clinical evaluation of tubule occlusion properties of toothpastes [25] using a conventional scanning electron microscope (SEM) and applying a replica technique to identify differences between positive and negative control DH toothpastes. SEM images of the impression of the dentine surface were independently scored by up to four individuals using a visual grading system. This model has now been further developed to use direct SEM imaging to successfully and reproducibly assess the occlusive effects of toothpastes for the relief of DH *in vitro* [17] and *in situ* [26–29].

In the present study, the tubule occlusion properties of an experimental toothpaste formulation containing 0.454% w/w SnF_2 were compared with those of a conventional fluoride toothpaste and a negative control (mineral water) over a 10-day period using a modification of the previously reported *in situ* model [26–30]. In addition, the robustness of any occlusion generated by the treatments was tested by exposing the dentine samples to an acid challenge (orange juice) after 9 and 10 days' treatment. The extent of tubule occlusion (surface coverage) was examined by direct SEM [27,28,30]. Modifications to the previously used *in situ* model included (i) the timeframe of the model, which was extended from the usual 4 days to 10 days of treatment; and (ii) the baseline score for each specimen was paired with its post-treatment score, thus each specimen provided its own control. The null hypothesis was that there is no difference in the occluding potential of a test and control toothpaste after 8 days of treatment.

2. Methods

This was a single-centre, single-blind (with respect to the dentine-sample analyst responsible for SEM and image grading), randomised, three-treatment, three-period, crossover, *in situ* study in healthy participants. The study was conducted in accordance with the Declaration of Helsinki at a UK Dental School, with ethical approval given by National Research Ethics Service (NRES) Committee South West – Exeter independent ethics committee (Research Ethics Committee [REC] Ref: 16/SW/0123). The study is registered at clinicaltrials.org; study number NCT02768194.

2.1. Participants

Participants were recruited by the study site. The eligible study population comprised healthy adults aged 18–80 years with good general and oral health and the ability to accommodate two lower bilateral buccal oral appliances, each fitted with four dentine samples. Exclusion criteria included pregnancy; breastfeeding; current or recurrent disease or dental pathology that could have affected study outcomes; current susceptibility to acid regurgitation; orthodontic appliances, restoration, bridgework or dentures that could have interfered with study assessments; recurrent or regular aphthous ulcers; severe gingivitis, carious lesions or periodontal disease; signs of severe dental erosion; any condition or medication that was causing xerostomia; and requirement for antibiotic prophylaxis for dental procedures.

2.2. Dentine samples and buccal appliances

During the study, participants wore lower left and lower right buccal appliances, each fitted with four dentine samples (eight samples in total), as described previously [25]. Dentine samples were obtained from recently extracted caries-free human third molars, donated to a Tooth Tissue Bank under ethical approval by NRES Committee Northern Ireland (REC Ref: 11/NI/0145). The roots were sectioned using a microslice (Ultra Tec Manufacturing Inc, Santa Ana, CA, USA) to obtain the dentine samples, which were then embedded in dental composite (QUIXFIL; Dentsply, Weybridge, UK). Each dentine sample was polished using a lapping and polishing machine (Kent 3 Automatic; Kemet International Ltd, Maidstone, UK) to produce a flat, level sample with parallel sides. The dentine samples were then etched with 10% citric acid for 30 s and washed in copious amounts of distilled water to expose the dentine tubules. Samples were stored in 0.9% saline solution until use.

Before use, each dentine sample was analysed using a non-destructive Phenom G2 pro desktop SEM (PhenomWorld, Eindhoven, The Netherlands) and the image was recorded. Each sample image was assessed by the sample-preparation technician for degree of tubule patency by visual grading using a categorical grading system (occlusion classification scale: 0 = not evaluable; 1 = occluded; 2 = mostly occluded; 3 = equally occluded/unoccluded; 4 = mostly unoccluded; 5 = unoccluded) [19]. Only dentine samples demonstrating minimal occlusion (≥ 4) were included in the study. SEM micrographs of the dentine samples deemed appropriate for use in the study formed the basis of the baseline occlusion classification score for each sample.

Following baseline SEM analysis, the samples were stored in 0.9% saline solution at 5 °C until placement in the buccal appliance. The dentine samples and appliance were disinfected before insertion in the mouth. When the dentine samples were removed from the appliances for SEM analysis at the end of Days 1, 4 and 8 (one from each appliance), a blank acrylic replicate of the dentine sample was inserted into the appliance to maintain a constant feel to the participant. Four new dentine samples were placed in the appliances for each treatment period.

2.3. Study products

Three study products were tested in separate treatment periods: Test toothpaste containing anhydrous 0.454% w/w SnF_2; Control fluoride toothpaste containing 0.76% w/w sodium monofluorophosphate (US Colgate® Cavity Protection; Colgate-Palmolive Company, New York, NY, USA); Volvic® mineral water (Danone, Paris, France). The study toothpastes were supplied in tubes over-wrapped in opaque vinyl with any branding obscured to maintain the study blind as far as possible. The mineral water (for negative control, rinsing and moist storage pots) was supplied in commercial bottles.

2.4. Study visits

The study comprised a screening visit followed by three treatment periods, each consisting of 10 non-consecutive treatment days (to be completed within 25 days of the first treatment day), with a wash-out period of at least 24 h (maximum 15 days) between treatment periods. At the screening visit, participants provided written informed consent and a medical history was taken. Participants underwent full oral soft tissue (OST) and hard tissue examinations and were screened for

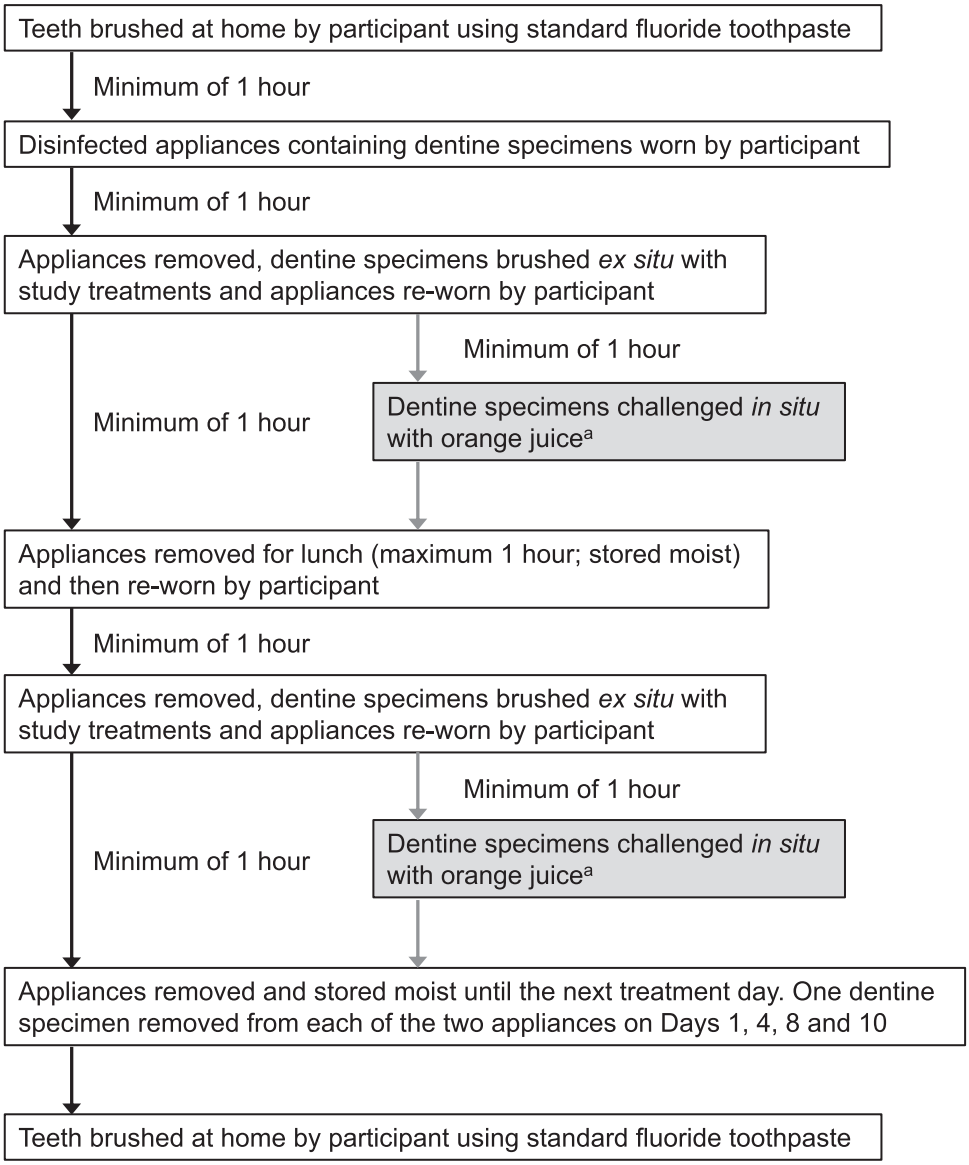

Fig. 1. Flow of procedures during treatment days.
[a]Procedure only conducted on Treatment Days 9 and 10.

eligibility. Eligible participants were provided with a standard fluoride toothpaste (US Colgate® Cavity Protection; Colgate-Palmolive Company, New York, NY, USA) and manual toothbrush (Aquafresh® Clean Control; GSK Consumer Healthcare, Weybridge, UK) and were instructed to brush twice-daily at home in their usual manner for the duration of the study. Participants were not permitted to use any other oral-care products, except for dental floss.

At the start of the first treatment period (between 1 and 15 days after the screening visit), eligible participants were randomised to the sequence in which their dentine samples received the three study treatments (one study product per treatment period). Randomisation numbers were assigned in ascending numerical order as each participant was determined to be fully eligible. The randomisation schedule used a balanced pair of Williams squares generated by the Biostatistics Department of GSK Consumer Healthcare.

The procedure flow on treatment days is summarised in Fig. 1. On treatment days, participants brushed with the standard fluoride toothpaste before attending the study site and brushed with it again in the evening after all study assessments had been completed. During the treatment day, each participant wore the oral appliances from 09:00 h ± 30 min to 15:30 h ± 30 min. On the morning of the treatment day, participants wore their appliances for at least an hour before the appliances were removed for *ex vivo* application of the study treatment.

Applications of study treatments were scheduled at 10:00 h ± 30 min and 14:30 h ± 30 min. Depending on the randomisation schedule, dentine samples retained within the appliances were immersed in either a slurry of freshly prepared treatment toothpaste (1:3 w/w in mineral water) or in mineral water alone. They were brushed *ex situ* by the study staff using an electric toothbrush (Oral B® Vitality Precision Clean power toothbrush with EB17 Precision Clean brush head; Procter & Gamble, Weybridge, UK) for 1 timed minute (2 min total for both of the participant's appliances; 15 s for each sample, including the acrylic substitutions).

The appliances were returned to the participant's mouth and the participant rinsed with 10 mL of mineral water for approximately 5 s and expectorated. The appliances were worn for at least 1 h before lunch. The appliances were removed from the mouth and stored in a moist pot for up to 1 h while the participants had lunch. This treatment schedule was repeated in the afternoon, ensuring first that the appliances were worn for at least 1 h following lunch, prior to treatment and

for at least 1 h following the second treatment of the day. As such, participants wore the appliances for at least 4 h on each treatment day. At the end of each treatment day, appliances were stored in a moist pot at the study site.

At the end of treatment days 1, 4, 8 and 10, one dentine sample was removed from each appliance (two samples in total per participant) for SEM analysis. On Days 9 and 10 of each treatment period, acid challenges were conducted at 11:30 h ± 30 min and 15:30 h ± 30 min (a minimum of 60 min after the morning and afternoon treatments, respectively). With both of the appliances *in situ*, the participants swished a total of 250 mL of orange juice around their mouth under supervision over a 10-minute period (25 mL/min), expectorating after approximately 1 min. Participants then rinsed their mouth for 5 s with 10 mL of mineral water. Following the afternoon acid challenge and mineral-water rinse (15:30 h ± 30 min), appliances were removed from the mouth and stored in moist pots at the study site.

2.5. Assessments

The extent of tubule occlusion was assessed by appropriately trained examiners grading SEM images at baseline, following days 1, 4, 8 and 10. The SEM used was capable of imaging without sputter-coating the samples with conductive material (standard for SEM methodologies to avoid charge build-up on the sample resulting in defocused images), thus enabling samples to be directly imaged to identify patent tubules prior to them being placed in the oral cavity. Images captured before intra-oral use were scored and paired with their post-treatment sample scores.

The approximate same area on the specimen surface was imaged at baseline and after treatment. Images were captured at ×2000 magnification. The pre- and post-treatment SEM images were independently scored according to the occlusion classification scale (as above) [17] by four trained, validated, blinded examiners and the scores for each specimen were paired. All examiners scored all images, which were presented to them in random order.

A calibration exercise was performed for dentinal tubule occlusion classification scoring of SEM images before study commencement. Four examiners graded the same 25 standard images obtained from a previous study. Assessments were compared with the calibration standard assessments. Each of the examiner's scores was cross-tabulated against the calibration standard. A weighted kappa coefficient (κ) was calculated using the Fleiss–Cohen method of weighting [31] to assess inter-examiner reliability and presented along with 95% confidence intervals (CIs). Reliability was deemed to be 'excellent' if $\kappa > 0.75$; 'fair to good' if $\kappa \geq 0.4$ and ≤ 0.75; and 'poor' if $\kappa < 0.4$. 'Excellent' reliability was required for an examiner to be selected.

2.6. Safety

Oral examinations were performed at screening, at the end of each treatment period and at a follow-up visit within 7 days of the final treatment visit or participant withdrawal. Adverse events (AEs) and any appliance incidents were recorded from the first use of standard fluoride toothpaste until 7 days after the end of the third treatment period. Safety was assessed from OST examination findings and AEs reported by participants following re-insertion of oral appliances in the mouth after treatment. All analyses of safety were made on the safety population, defined as all participants who were randomised and had the oral appliances re-inserted in the mouth after *ex vivo* treatment application. The safety population was analysed as per treatment received.

2.7. Statistical analysis

At least 24 eligible participants were planned to be randomised to ensure approximately 20 completed the entire study. With 20 participants, using a paired *t* test, a treatment difference of 0.69 units could be detected with 80% power, assuming 1.1 as the variance of paired differences. The estimate for variance was obtained from an earlier study [29] where all residual variances were < 0.55. The intent-to-treat (ITT) population was defined as all randomised participants with at least one graded post-treatment SEM image. The primary population for analysis was the per-protocol (PP) population, defined as those in the ITT population for whom all post-baseline SEM image scores were deemed to be unaffected by protocol violations. For one participant, the Period 1, Day 10 SEM data were deemed to be affected by a protocol violation (samples being outside the mouth for a longer duration than specified), resulting in exclusion of data for this time-point only.

The primary efficacy variable was change from baseline in mean occlusion classification score. This was calculated separately for each sample as the mean of the values from the independent classifications of the four examiners. The mean was calculated only if at least two of the four examiners assigned a score > 0. The primary objective was to evaluate the model's ability to differentiate the dentinal tubule occlusion properties between the Test toothpaste and water (negative control) after 8 days of treatment. The secondary objective was to evaluate the model's ability to differentiate the dentinal tubule occlusion properties of the toothpaste formulations after 1, 4 and 10 days' treatment.

The change from baseline in the mean occlusion classification score was analysed using a mixed-model repeated-measures (MMRM) analysis with participant included as a random factor, and treatment, period, day, side of the mouth and treatment × day interaction included as fixed factors; baseline mean SEM score was the covariate. Participant (period) was the unit for the repeated measure analysis and the specified covariance matrix was the Kronecker product of a 4 × 4 unstructured matrix and a 2 × 2 matrix with compound symmetry corresponding to the 4 days in each period and the two sides of the mouth for each day. This model was used to compare treatments at the 5% level at 8 days using appropriate contrasts for the factors of treatment and treatment × day; p-values and 95% CIs were tabulated for the differences. The same MMRM model was used to compare treatments at 1, 4 and 10 days using corresponding appropriate contrasts for the factors of treatment and treatment × day. The assumption of normality and homogeneity of covariance for residuals was investigated and found to be acceptable. Note that no adjustment for multiple comparisons was made as a primary efficacy assessment had been pre-specified.

3. Results

A total of 26 participants were screened, 24 were randomised to study treatments and 21 completed all three treatment periods (Fig. 2). The first participant was enrolled on 6 July 2016; the last participant completed the study on 21 September 2016. All randomised participants were included in the safety population (n = 24), the mean age of whom was 41.4 years (range 20–61 years; standard deviation 13.31 years). The majority of participants were female (13/24 [54.2%]). No participants were excluded from the PP population due to protocol violations. Kappa scores for the four examiners were very similar (Examiner 1: 0.93 [95% CI 0.89, 0.99]; Examiner 2: 0.92 [0.85, 0.98]; Examiner 3: 0.90 [0.79, 1.00]; Examiner 4: 0.87 [0.75, 0.99]). Reliability of all four was deemed to be 'excellent' and all four subsequently graded the study SEM images.

3.1. Efficacy

The raw mean occlusion scores of the dentine samples at baseline and following removal from the appliances after 1, 4, 8 or 10 days are provided in Table 1 for each treatment. Fig. 3 summarises the change from baseline in the mean occlusion scores over time by treatment. The results of the between-treatment analyses are summarised in Table 2.

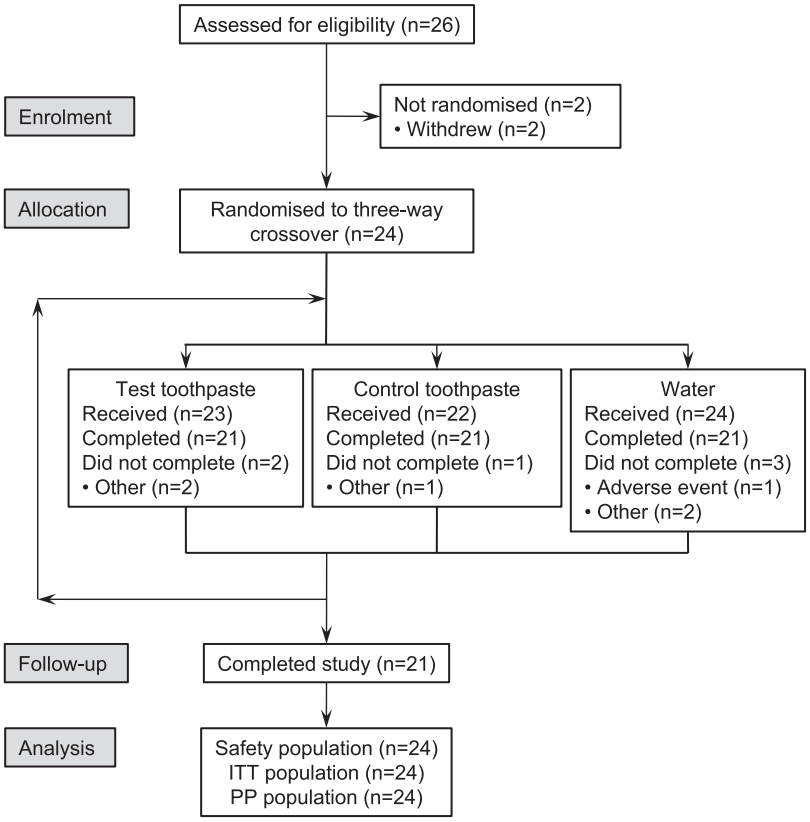

Fig. 2. Flow chart of participant disposition.
Footnote: 'Other' reasons for non-completion of the treatment period included reasons other than not meeting the study criteria, experiencing adverse events, being lost to follow-up, protocol deviation or withdrawal by the participant. ITT, intent-to-treat; PP, per-protocol.

Table 1
Mean tubule occlusion score in dentine samples before and after treatment (per-protocol population).

	Test toothpaste (N = 23)		Control toothpaste (N = 22)		Water (N = 24)	
Timepoint	Before (n = 46)	After (n = 46)	Before (n = 44)	After (n = 44)	Before (n = 48)	After (n = 48)
Day 1	4.30 (0.062)	3.96 (0.085)	4.38 (0.055)	4.13 (0.116)	4.30 (0.070)	3.86 (0.139)
Day 4	4.41 (0.069)[a]	3.51 (0.123)[a]	4.30 (0.070)	3.60 (0.127)	4.23 (0.058)	3.98 (0.110)
Day 8	4.38 (0.056)	3.43 (0.112)	4.32 (0.078)	3.42 (0.114)	4.36 (0.060)[b]	3.53 (0.130)[b]
Day 10	4.31 (0.062)[b]	3.13 (0.144)[b,c]	4.31 (0.063)	3.49 (0.127)[c]	4.36 (0.066)[d]	3.74 (0.133)[c,d]

Data are raw means (standard error). N, number of participants; n, number of dentine samples.
[a] n = 45 (one sample missing).
[b] n = 44.
[c] After 2 days of acid challenges.
[d] n = 42.

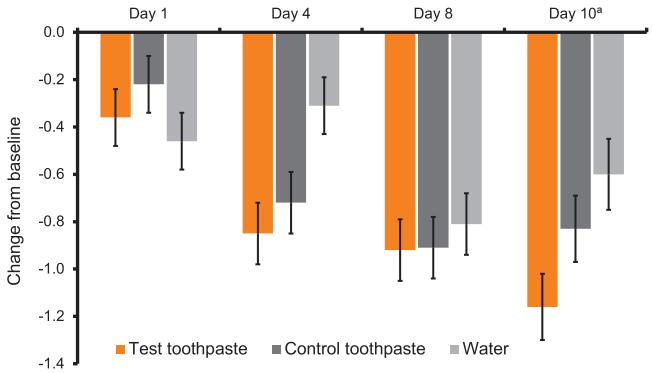

Fig. 3. Mean change in tubule occlusion score from baseline (per-protocol population).
Data shown are adjusted means ± standard errors. [a]Following 2 days of acid challenges. [Greyscale figure can be used for print]

While the occlusion scores for the dentine samples treated with either the Test or the Control toothpaste reduced (*i.e.*, degree of occlusion increased) with time, there were no statistically significant differences in occlusion scores between any of the treatment groups at Day 8 (the primary efficacy measure). At Day 4, compared with the samples treated with water, there was a statistically significantly greater degree of occlusion for the dentine samples treated with either the Test or the Control toothpaste ($p = 0.0023$ and $p = 0.0194$, respectively); however, there was no statistically significant difference between the two toothpaste groups at this timepoint. Following the acid challenges (Day 10), there was a statistically significantly greater degree of occlusion in the Test toothpaste-treated dentine samples *versus* those treated with water ($p = 0.0058$). No other comparisons were statistically significant.

3.2. Safety

Eleven treatment-emergent AEs were reported by six participants; of these, two were oral (tooth sensitivity and tooth fracture) and nine were non-oral. All treatment-emergent AEs were mild in intensity except for the tooth fracture, which was of moderate intensity. All AEs resolved by the end of the study. One participant was withdrawn from the study due to the tooth-sensitivity AE, which was ascribed to exposure to the acid challenge (orange juice) used in the study. No treatment-related AEs, no

Table 2
Between-treatment comparisons[a] of mean occlusion scores (per-protocol population).

Day	Test toothpaste *vs* water		Test toothpaste *vs* Control toothpaste		Control toothpaste *vs* water	
	Difference[b] (95% CI)	p-value	Difference[b] (95% CI)	p-value	Difference[b] (95% CI)	p-value
1	0.10 (-0.23, 0.42)	0.5573	−0.14 (-0.47, 0.19)	0.4044	0.23 (-0.09, 0.56)	0.1569
4	−0.54 (-0.87, -0.20)	**0.0023**	−0.13 (-0.47, 0.22)	0.4683	−0.41 (-0.75, -0.07)	**0.0194**
8	−0.11 (-0.46, 0.25)	0.5512	−0.01 (-0.36, 0.35)	0.9671	−0.10 (-0.46, 0.26)	0.5830
10[c]	−0.56 (-0.96, -0.17)	**0.0058**	−0.33 (-0.72, 0.06)	0.1007	−0.24 (-0.64, 0.16)	0.2430

Values in bold indicate statistically significant difference at $p < 0.05$.
CI, confidence interval.
[a] From mixed-model repeated-measures analysis with treatment, period, day, side of mouth, treatment × day interaction as fixed factors, participant as a random effect and baseline scanning electron microscope image score as a covariate. Participant within period was the unit for the repeated measure.
[b] First-named treatment minus the second-named treatment, such that a negative difference favours the firstnamed treatment.
[c] After 2 days of acid challenges.

serious AEs, and no medical device incidents were reported.

4. Discussion

Stannous ions have previously been found effective in reducing the pain of DH in clinical studies when formulated into toothpastes in the form of SnF_2 [28,32–34] and stannous chloride combined with sodium fluoride [35]. The exploratory SnF_2 toothpaste used in this study has been shown in clinical trials to reduce the severity of DH pain compared with conventional fluoride toothpaste in studies ranging from single brushing to 14 days' twice-daily brushing [22–24]. Similar formulations have shown benefits after 4 and 8 weeks of use [28,29].

In vitro studies have shown that SnF_2 in toothpaste forms a surface coating over the dentine and penetrates into the dentine tubules [19]. The development of an *in situ* model that allows direct and non-destructive imaging of the dentine surface before and after treatment has allowed a greater understanding of how components in toothpastes interact with the dentine surface in a clinical situation [25,28,29]. Direct imaging of the surface, or even below the surface, of dentine samples allows visualisation of the physical interaction and the level of tubular occlusion that leads to the reduction of DH identified during clinical studies. The ability to score dentine images before inclusion in an *in situ* study should strengthen the outcome of a study as each specimen acts as its own control.

The modified *in situ* model used here has not previously been correlated with short-term SnF_2 DH studies so the purpose was to explore whether this model reflected the tubule-occluding properties of SnF_2 toothpastes observed in clinical studies. A similar model has previously been able to demonstrate dentinal tubule occlusion by toothpastes formulated with other occluding agents when run for a total of 4 days (2 days without acid challenges, then 2 days with acid challenges) [25]. The same model has also shown statistically significantly greater occlusion for a similar SnF_2 formulation to the one in the present study when compared with the same control toothpaste after 2, 3 and 4 days [28,29]. Of note, the water and control toothpaste in the latter study [29] gave occlusion scores that were essentially constant for all treatment days, in contrast to the variable scores seen across treatment days in this study.

In vitro studies using SEM have demonstrated the ability of stannous ions to occlude dentine tubules in laboratory conditions. A study of two SnF_2-containing toothpastes – one aqueous, one non-aqueous – showed they were more effective in occluding dentinal tubules by both visual analysis of SEM images and hydraulic conductance, than a regular toothpaste. This difference was maintained after acid treatment [20]. Another study, which used focused ion-beam SEM to image a cross-section of the dentine tubules, showed that a SnF_2 dispersion in glycerol and an experimental anhydrous SnF_2 toothpaste both created an occlusive layer that extended into the tubules below the dentine surface [23].

The principal modification made to the model for the current study was to increase the duration of application from 4 days to 8 days, with a further 2 days with an acid challenge. This was to evaluate whether a longer duration of application would facilitate development of a visible (to the SEM) occluding layer, leading to larger treatment differences as previously seen when the model was extended to investigate the occlusion properties of a toothpaste containing calcium sodium phosphosilicate (CSPS; Novamin®) [30].

Here, there was no significant difference between groups after 1 day of application, but a significant difference between the toothpaste treatment groups and the water treatment group was observed after 4 days of application. This difference was not observed after 8 days of treatment application, when the water treatment group showed a higher degree of occlusion compared with the Day 4 measurement. A significant difference was shown between the Test toothpaste and water again at Day 10 following the acid challenges. The treatment effects observed with the Control toothpaste and water control in this study were much greater than those seen previously [26,27]. This observation appears to demonstrate an inherent variability in this clinical methodology, mirrored by the greater than expected variance in the mean scores, and probably reduced observed treatment differences. The increased level of occlusion for the Control and water groups in this study may be as a result of the increased time the appliances resided in the mouth, allowing more debris, such as salivary constituents and bacterial plaque, to build up on the dentine surface. Given this possibility, a recommendation for future studies would be to incorporate acid challenges during every day of treatment application.

Care must be taken when comparing results from *in situ* clinical and laboratory studies of occlusion as they use different measuring instruments; some may determine tubular occlusion by looking directly at the surface, while other instruments may focus on the sub-surface area. Similarly, extrapolating results observed in a clinical study to those observed in an *in vitro* or *in situ* study may also be misleading.

A potential drawback of the approach used in this study to quantify and visualise tubule occlusion is that the surface coverage observable by SEM, and occlusion of dentine tubules that prevents fluid flow, are not the same phenomenon. Evidence from *in vitro* studies suggests that the deposition critical to tubule occlusion with SnF_2, and thus responsible for DH relief, may be within the dentine tubules rather than at the dentine surface [23] and so not visible by SEM. The results from this study strengthen this theory, because viewing the dentine surface from above did not show any significant difference in the degree of occlusion from the SnF_2 containing toothpaste after 8 days of treatment. Clinical studies using the same toothpaste have shown it to provide relief from DH [22–24]. The model may therefore need to be further modified to include cross-sectional imaging of the tubules to identify occluding particles further down inside them. It must also be stated that a further limitation of this model is that an *in situ* model does not allow fluid flow through the tubules in the same way that is found clinically. The fluid flow within tubules may be essential to form precipitates following treatment with SnF_2, more so than other agents that have been shown to occlude the surface when viewed under SEM using this type of occlusion model. Studies on another occlusion technology – CSPS over 10 and 20 days – have suggested that the precipitated layer overlying the tubules is more substantial and may play a larger role in prevention of fluid flow in the tubules *versus* intra-tubular precipitation [30,19]. Treatment differences were observed to be greatest in numerical terms at Day 10, consistent with clinical data indicating that degree of DH

relief increases with duration of use over this time period [22,23].

5. Conclusions

This modified *in situ* model was unable to demonstrate statistically significant differences in dentinal tubule occlusion by SEM of a SnF_2 toothpaste compared with a conventional control toothpaste after 8 days' use, therefore the null hypotheses cannot be rejected. Further developments have been identified for this *in situ* model that can be made to improve differentiation between products. Study treatments were generally well-tolerated.

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Author contributions and disclosure statement

Study design involved SBJ, NXW, JS, ELM, NH. Study conduct and data acquisition involved NXW, ELM, JS, NH, SBJ. Data interpretation was led by NXW and JEC, and involved all authors. All authors contributed to the drafting of the manuscript and approved the final copy. The study was supported by GSK Consumer Healthcare, of which J.E. Creeth is an employee. N.X. West, *J*. Seong, N. Hellin, E.L. Macdonald and S.B. Jones are employees of the Division of Restorative Dentistry (Periodontology), Bristol Dental School and Hospital, which has received funding from GSK Consumer Healthcare.

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